



# Isolating Genomic DNA from Whole Blood using Thermo Scientific™ M Series centrifuges

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## Abstract

The collection of pure high molecular weight genomic DNA from whole blood or cell cultures is a key starting step for researchers conducting downstream research such as PCR amplification or restriction digests. In this protocol, high purity DNA was collected from the buffy coat layer of human blood from 10 patients for downstream analysis. Using the Nucleon® BACC2 kit, DNA results were analyzed to prove acceptable yield and purity in the Thermo Scientific M Series refrigerated microcentrifuge with 24-place 1.5/2.0 ml rotor. Readily available kits, such as the Nucleon BACC2 from GE® Cytiva Health Care Europe-Amersham, minimize the need for reagents and consumables. The end-user only has to help to provide a high quality refrigerated microcentrifuge and pipettes to complete the isolation process. It is important to find a suitable DNA isolation system and to use appropriate equipment to satisfy downstream applications, including enzyme applications and cDNA synthesis. The Thermo Scientific M Series 24-place refrigerated microcentrifuge proved to be

ideal for use with DNA extraction kits such as the Nucleon BACC2 kit.

## Materials and Methods

Human blood samples were collected from 10 patients. DNA was extracted from the buffy coat layer, following the instructions included with the Nucleon BACC2 kit from GE Cytiva Health Care Europe-Amersham. The protocol described in this application note is a summary of the procedure included in the kit manual provided by the manufacturer. Refer to the kit manual for detailed product information and protocols. All centrifugation steps were performed using the Thermo Scientific refrigerated microcentrifuge with the standard 24-place 1.5/2.0 ml rotor at 4°C.

## Procedure

### Cell lysis

1. In a 1.5 ml microcentrifuge tube, resuspend 400 µl of the buffy coat in 1 ml of solution A (sodium perchlorate).
2. Pellet the cells in the refrigerated microcentrifuge's 24-place rotor by centrifuging at 6000 rpm for 5 minutes at 4°C.
3. Discard the supernatant and resuspend the cell pellet 2 or 3 times in 700 µl of solution A (sodium perchlorate).

### Deproteinization with sodium perchlorate

4. Discard the supernatant and resuspend the cell pellet in 500 µl of reagent B (chloroform) with brief vortexing.
5. Add 125 µl of sodium perchlorate and invert the tube several times.

### Extraction with chloroform and Nucleon resin

6. Add 500 µl of chloroform and invert the tube for 20-30 seconds.
7. Add 150 µl of Nucleon silica and centrifuge at 6500 rpm in the refrigerated microcentrifuge for 3 minutes at 4°C.

### DNA precipitation

8. Transfer the upper phase (500 µl) to a fresh 1.5 ml tube and precipitate the DNA with 2 volumes of cold absolute ethanol.
9. Centrifuge at 12,000 rpm in the Thermo Scientific refrigerated microcentrifuge for 10 minutes at 4°C.

### DNA washing

10. Wash the DNA with 1 ml of 70% cold ethanol.
11. Pellet the DNA again, centrifuging at 12,000 rpm in the Thermo Scientific refrigerated microcentrifuge for 10 minutes.
12. Air dry and resuspend the DNA in TE buffer.



Thermo Scientific™ M Series microcentrifuges with polypropylene rotor

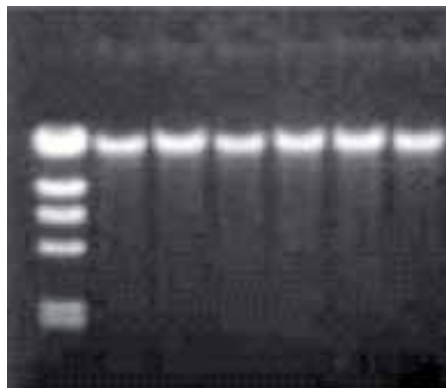
### Evaluation of nucleic acid purity

Optical density measurements were used to assay the DNA yield and check for contamination by salts, solvents and proteins. To evaluate the purity of the extracted DNA, absorbance ratios at 260 nm/230 nm (DNA/Organic or carbohydrates contaminants like phenol and other aromatic compounds) and 260 nm/280 nm (DNA/protein) were determined. (A typical yield is 200- 400 µg of DNA with a 260/280 ratio of 1.8 to 2.)

## Results

### Evaluation of DNA purity

Optical density measurements were taken after DNA extraction using the Nucleon BACC2 kit and the Thermo Scientific refrigerated microcentrifuge. An OD 260/280 ratio of 1.8 to 2 and an OD 260/230 ratio of 1.8 or greater indicate that good quality DNA was obtained (see Table 1, Figure 2). Pure DNA is then ready for downstream processes.



**Figure 2.** DNA isolated from human blood following the Nucleon protocol kit and using the Thermo Scientific refrigerated microcentrifuge. DNA was separated on a 1 % agarose gel. Lane 1 contains 1 x Hind III molecular weight markers, lanes 2-7 contain samples.

### Conclusion

High yield, high purity DNA was obtained from human blood using the Nucleon BACC kit system with the Thermo Scientific refrigerated microcentrifuge and 24-place 1.5/2.0 ml rotor. DNA prepared in this way is ready to be digested with restriction endonucleases and has been employed for DNA profiling and real-time PCR. The user-friendly Thermo Scientific M Series refrigerated microcentrifuge with 24-place 1.5/2.0 ml rotor has shown to be an outstanding performer for constant stable refrigeration and is recommended for all laboratories using standard methods or kits for isolation of genomic DNA or other kit protocols.

### References:

GE Health Care Europe-Amersham; Product Codes RPN 8501, 8502 or 8512; Nucleon BACC, Genomic DNA from Blood and Cell Cultures publication number 18-1146-65, Rev 1.

Sample ID	Quantity ng/µl	Sample ID	Sample ID
Patient 1	476.71	1.87	2.11
Patient 2	534.07	1.88	1.96
Patient 3	546.04	1.88	1.83
Patient 4	791.17	1.92	1.84
Patient 5	805.83	1.91	1.90
Patient 6	611.87	1.89	1.80
Patient 7	695.31	1.90	1.83
Patient 8	533.25	1.87	1.88
Patient 9	562.41	1.87	1.84
Patient 10	648.24	1.90	1.83

Specifications	M17 Series	M21 Series
<b>Max RCF</b>	17,000 xg	21,100 xg
<b>Max Speed</b>	13,300 rpm	14,800 rpm
<b>Max Noise Level</b>	<b>MT 17:</b> <54db(A)(Rotor 24x2ml; 13300 RPM) <b>MTR 17:</b> <48db(A)(Rotor 24x2ml; 13300 RPM)	<b>MT 21:</b> <56db(A)(Rotor 24x2ml; 14800 RPM) <b>MTR 21:</b> <56db(A)(Rotor 24x2ml; 14800 RPM)
<b>Weight</b>	<b>MT 17/MT 21:</b> 120V/230V 11 kg; <b>100V:</b> 12kg <b>MTR 17/MTR 21:</b> 120V 24 kg; 230V 24kg/25kg <b>100V:</b> 25kg	
<b>Acceleration/Deceleration Rates</b>	11/12 seconds (ventilated); 10/12 seconds (refrigerated)	
<b>Time Set Range</b>	1 min - 99 min; 1 min increments + HOLD mode	
<b>Temperature Range<sup>4</sup></b>	Set from -9 °C to +40 °C per 1 °C increment	
<b>Certifications</b>	CSA certified, CE marked, IVD compliant, Certified biocontainment <sup>1</sup>	
<b>Technical Standards</b>	IEC 61010-1, IEC 61010-2, IEC 61010-020	
<b>Warranty</b>	2 years	
<b>Dimensions (H X W x D)</b>		
<b>MT 17 and MT 21</b>	225 x 243 x 352 mm (8.86 x 9.56 x 13.8 in)	
<b>MTR 17 and MTR 21</b>	330 x 290 x 440 mm (12.9 x 11.4 x 17.3 in)	

<sup>1</sup>Biocontainment certification by Public Health England, Porton Down, UK. <sup>4</sup>M17 series centrifuges only.

Ordering information	MT 17		MT 21		MTR 17		MTR 21	
	IVD-MD	General Use Only	IVD-MD	General Use Only	IVD-MD	General Use Only	IVD-MD	General Use Only
120 V, 50/60 Hz	75002576	N/A	75002579	N/A	75002588	N/A	75002591	N/A
230 V, 50/60 Hz	75002577	75002571	75002580	75002574	75002589	75002583	75002592	75002586
100 V, 50/60 Hz	N/A	N/A	75002581	75002575	N/A	N/A	75002593	75002587
230 V, 60 Hz for USA only	N/A	N/A	N/A	N/A	75002597	N/A	75002599	N/A

All part numbers include a 24 x 1.5/2 mL rotor

Application Package	Voltage	Accessories included	IVD-MD or General use	Cat. No.
Everylab Centrifuge Package <b>MT 21</b>	120V	incl. 24 x 1.5/2 mL rotor, Thermo Scientific™ Finnpiptette™ F2 GLP Kit 2: <ul style="list-style-type: none"> <li>Single-channel 0.2-2 µL, 2-20 µL, 20-200 µL and 100-1,000 µL pipettes</li> <li>Flex tips: 10 micro: 1 x 96; 200: 2 x 96; 1000: 1 x 96</li> <li>F-stand • Reagent reservoir demo pack and</li> <li>1.5 Snap Cap Low Retention microcentrifuge tubes, full case (5,000 tubes)</li> </ul>	General use	75002532
Everylab Centrifuge Package <b>MTR 21</b>	120 V	incl. 24 x 1.5/2 mL rotor, Thermo Scientific™ Finnpiptette™ F2 GLP Kit 2: <ul style="list-style-type: none"> <li>Single-channel 0.2-2 µL, 2-20 µL, 20-200 µL and 100-1,000 µL pipettes</li> <li>Flex tips: 10 micro: 1 x 96; 200: 2 x 96; 1000: 1 x 96</li> <li>F-stand • Reagent reservoir demo pack and</li> <li>1.5 Snap Cap Low Retention microcentrifuge tubes, full case (5,000 tubes)</li> </ul>	General use	75002533
<b>MT 17</b>	230 V, 50/60 Hz	includes hematocrit rotor	IVD	75002578
<b>MT 17</b>	120 V, 50/60 Hz	includes hematocrit rotor	IVD	75002598
<b>MT 21</b>	120 V, 50/60 Hz	includes 24 x 1.5/2.0 mL rotor and 18x2 0/0.5 mL rotor	IVD	75002584
<b>MTR 21</b>	230 V, 50/60 Hz	includes 24 x 1.5/2.0 mL rotor and 18x2 0/0.5 mL rotor	General use	75002596
<b>MTR 21</b>	120 V, 50/60 Hz	includes 24 x 1.5/2.0 mL rotor and 18x2 0/0.5 mL rotor	IVD	75002590
<b>MTR 21</b>	230 V, 50/60 Hz	includes 24 x 1 5/2.0 mL rotor and 18x2 0/0.5 mL rotor	IVD	75002594

Learn more at [thermofisher.com/centrifuges](https://thermofisher.com/centrifuges)

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